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## Diagnostic strategy and long-term treatment outcomes in Idiopathic Pulmonary Arterial Hypertension

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## Chapter 5

### **The right ventricle explains sex differences in survival in idiopathic pulmonary arterial hypertension**

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## Abstract

### *Background*

Male sex is an independent predictor of worse survival in pulmonary arterial hypertension (PAH). This finding might be explained by more severe pulmonary vascular disease, worse right ventricular function or different response to therapy. The aim of this study was to investigate the underlying cause of sex differences in survival in treated PAH patients.

### *Methods*

This was a retrospective cohort study of 101 patients with PAH (82 idiopathic, 15 heritable, 4 anorexigen associated) who were diagnosed at our institute between February 1999 and January 2011 and underwent right heart catheterisation (RHC) and cardiac magnetic resonance imaging (CMR) to assess right ventricular function. Change in pulmonary vascular resistance was taken as a measure of treatment response on the pulmonary vasculature, whereas change in right ventricular ejection fraction was used to assess right ventricular response to therapy.

### *Results*

Pulmonary vascular resistance and right ventricular ejection fraction were comparable between men and women at baseline, however male patients had a worse transplant-free survival compared to female patients ( $p=0.002$ ). While male and female patients showed a similar reduction in PVR after one year, RVEF improved in female patients whereas it deteriorated in male patients. In a mediator analysis, after correcting for confounders, 39.0 % of the difference in transplant-free survival between men and women was mediated through changes in RVEF after initiating PAH medical therapies.

### *Conclusions*

This study suggests that differences in RVEF response with initiation of medical therapy in IPAH explain a significant portion of the worse survival seen in males.

## Introduction

Pulmonary arterial hypertension (PAH) is a rare disease characterized by obstructive lesions of the small pulmonary vessels, leading to increased pulmonary artery pressure (PAP), right-sided heart failure and death within several years.<sup>1-2</sup> Despite the advent of improved therapies outcome remains poor.<sup>3,4</sup> Prognosis correlates with severity of right ventricular (RV) structure and function.<sup>2,5</sup> More recently, male sex was identified as an independent predictor of mortality.<sup>6-10</sup> Men treated with endothelin receptor antagonists had less six minute walk improvement.<sup>11</sup> The cause of these sex differences is unknown, however a distinct vascular and/or right ventricular response to medical therapies is one possibility. Considering the need for improved treatments and “personalized therapy”, a better understanding of these sex differences would be important. The aim of our study was to investigate the role of the pulmonary vasculature and the right ventricle in explaining sex differences in survival of treated IPAH.

## Methods

### *Study design and patients*

All idiopathic (IPAH), anorexigen associated (APAH) and heritable PAH (HPAH) treated at the VU University Medical Center (VUMC) between February 1999 and January 2011 were eligible. Diagnosis was according to the guidelines and included right heart catheterisation (RHC). Medical treatment comprised prostacyclin analogues, endothelin receptor antagonists and phosphodiesterase type-5 inhibitors either alone or in various combinations. Patients with a positive vasodilator challenge were treated with calcium antagonists.<sup>1</sup> This was a retrospective cohort study of patients enrolled in an ongoing prospective study to assess the clinical value of cardiac magnetic resonance imaging (CMR) in PAH. All patients who had RHC and CMR performed prior to initiation of medical therapy (N = 101 out of N= 186 patients evaluated during this period) were included.

### *Right Heart Catheterisation*

Hemodynamic assessment was performed with a 7-F balloon tipped flow directed Swan-Ganz catheter (131HF7, Baxter Healthcare Corp. , Irvine, California). Baseline and follow-up RHC measurements of pulmonary artery pressure (PAP), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP), and cardiac output (CO) were obtained. Pulmonary vascular resistance (PVR) was calculated as  $(80 \cdot (\text{mean PAP} - \text{PCWP}) / \text{CO})$ . Vasoreactivity testing was with inhaled nitric oxide (20 ppm). Acute vasoreactivity defined as a mean PAP reduction  $\geq 10$  mmHg to reach an absolute value  $\leq 40$  mmHg with increased or unchanged cardiac output.

### *Cardiac Magnetic Resonance Imaging*

CMR was performed on a Siemens Avanto 1.5 T and 1.5 T Sonata scanner (Siemens Medical Solutions, Erlangen, Germany), equipped with a 6-element phased-array coil. ECG-gated cine imaging was performed using a balanced steady, free precession pulse sequence, during repeated breath-holds. Short-axis slices were obtained with, slice thickness 5 mm and interslice gap 5 mm, fully covering both ventricles from base to apex. Temporal slice resolution between 35 and 45 ms, voxel size 1.8 x 1.3 x 5.0 mm<sup>3</sup>, flip angle 60°, receiver bandwidth 930 Hz/pixel, TR/TE 3.2/1.6 ms, matrix 256 x 156.

End-diastolic and end-systolic endocardial and epicardial contours were delineated manually by an observer blinded to other clinical information and processed using MASS software (Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands) to obtain right ventricular end-diastolic and end-systolic volumes (RVEDV and RVESV respectively) and RV mass. Papillary muscles and trabeculae were excluded from the cavity, and included in RV mass. Right ventricular stroke volume (RVSV) and ejection fraction (RVEF) were calculated:  $RVSV = RVEDV - RVESV$  and  $RVEF = RVSV / RVEDV$ .<sup>12</sup> RV mass / RVEDV was used as a measure of relative RV wall thickness.<sup>13,14</sup>

### *Data analysis*

Measurements are reported as mean  $\pm$  standard deviation and median (interquartile range) where appropriate. Continuous variables were compared using student t-tests or Mann-Whitney U, where not normally distributed. Categorical variables compared using Pearson Chi-square tests and Fisher's exact tests, as needed.

Follow-up was until September 2011. Transplant-free sex survival differences were confirmed using Kaplan Meier curves and logrank test. Confounders accounted for by Cox regression. Variables leading to a  $\geq 10\%$  change in the coefficient for sex were included in the final survival prediction model. Variables screened for confounding included: age, height, weight, WHO functional class, number of comorbidities (1, 2 and  $\geq 3$ ), RVEF, RV wall thickness, glomerular filtration rate (GFR, Cockcroft), PVR and type of medical therapy used (prostacyclin yes/no, endothelin receptor antagonist yes/no and phosphodiesterase type 5 inhibitor yes/no).

Sex differences in secondary treatment outcomes (Nt-proBNP, 6-minute walk distance and renal function), RHC hemodynamics and CMR were confirmed using linear regression with the follow-up measurement as the dependent variable and the baseline measurement and sex as independent variables. WHO class change differences were confirmed by ordinal regression. Multiple imputation was used for missing follow-up MRI variables. We multiply imputed 100 datasets. Linear regression models were estimated in each dataset and regression coefficients and standard errors pooled and the p-value of each coefficient in the model determined. To correct for confounders a similar approach was used as discussed above for the survival analysis.

An exploratory mediator analysis was done to confirm that transplant-free sex survival differences were mediated through differences in RVEF change. Analysis was done according to Baron and Kenny<sup>15</sup> and consists of 3 steps. In step 1, sex was confirmed

as an independent predictor of transplant-free survival by Cox regression. Step 2 was to confirm that sex was an independent predictor of the proposed mediator by linear regression. Step 3 employs a Cox regression model for transplant-free survival including sex and the potential mediator as independent variables and its purpose is to confirm the proposed mediator is a significant predictor of survival, while controlling for sex. RVEF and PVR changes were both examined as potential mediators. This was done by adding follow-up measurements of respectively RVEF and PVR to a Cox regression equation containing gender and the baseline value. A greater than 10% change in the coefficient of sex after adding the follow-up value of the proposed mediator was accepted as evidence of significant mediation. The magnitude of the indirect (mediated) effect was calculated according to the following formula:

$$\text{Indirect effect} = 1 - (c' / c)$$

In the formula,  $c$  is the coefficient for sex in the Cox regression formula predicting survival, corrected for baseline RVEF;  $c'$  is the coefficient for sex in the Cox regression formula predicting survival corrected for RVEF baseline value and RVEF change by adding the follow-up RVEF value to the equation. In addition a mediator analysis corrected for all potential confounders mentioned earlier was performed.<sup>16</sup>

Analysis were performed using IBM SPSS statistics 19.0 software. This study was approved by the VUMC Research and Ethics Review boards (METC), and informed consent obtained. (approval number 2012288)

## Results

### *Patient characteristics and treatments*

One-hundred-eighty-six patients (155 IPAH, 25 HPAH and 6 APAH) were treated at the VUMC between February 1999 and January 2011. Eight-five patients were excluded. Reasons for exclusion were: no MRI due to logistical reasons ( $n=44$ ), first-line treatment elsewhere ( $n=25$ ), contraindications for MRI ( $n=11$ ) and no PAH medication initiated ( $n=5$ ). Apart from age table 1 indicates similar characteristics compared to those included for further analysis ( $n=101$ ). The six-minute walk tended to be greater in those included, however the % predicted distance was similar. The remaining 101 patients all had CMR and RHC at baseline before starting PAH specific medical therapies (table 2). In these patients men had larger RVEDV and RVESV, but had similar invasively measured hemodynamics and similar RVSV and RVEF compared to women. Median (IQR) time between baseline CMR and RHC was 0.2 (0.0-1.95) months. Table 3 depicts prescribed medications between baseline and follow-up assessment. Follow-up CMR and RHC were performed after respectively 1.1 (0.9-1.7) and 1.1 (0.9-2.2) years. Time on PAH specific medication was 5.4 (2.1-7.7) years. Time to addition of other PAH specific therapy was 5.0 (2.3-6.0) months for those patients who had PAH specific drugs added before follow-up measurements.

**Table 1.** Patient characteristics and Right Heart Catheterisation (RHC) measurements in Pulmonary Arterial Hypertension included in study (n=101) compared to those excluded (n=85)

|  | n=101       | n=85        |
|--|-------------|-------------|
| Age, years                                   | 48 ± 16     | 57 ± 18     |
| Gender, m/f                                  | 26/75       | 28/57       |
| BMI, kg/m <sup>2</sup>                       | 26 ± 5      | 28 ± 6      |
| WHO FC, n                                    |             |             |
| Class I                                      | 3           | 1           |
| Class II                                     | 14          | 19          |
| Class III                                    | 41          | 53          |
| Class IV                                     | 27          | 28          |
| Comorbidities, n                             |             |             |
| 0  | 34          | 30          |
| 1  | 32          | 22          |
| 2  | 20          | 19          |
| ≥ 3  | 15          | 14          |
| 6MWD, m                                      | 362 ± 162   | 307 ± 126   |
| 6MWD, % predicted                            | 61 ± 24     | 58 ± 21     |
| Creatinine, mmol/L                           | 98 ± 21     | 100 ± 23    |
| GFR, ml/min                                  | 78 ± 24     | 76 ± 31     |
| NT-proBNP, ng/L*                             | 1765 ± 1865 | 1824 ± 2486 |
| RHC  |             |             |
| RAP, mmHg                                    | 9 ± 5       | 9 ± 6       |
| mPAP, mmHg                                   | 56 ± 14     | 49 ± 12     |
| PCWP, mmHg                                   | 8 ± 5       | 10 ± 7      |
| CO, L/min                                    | 4.60 ± 1.63 | 4.65 ± 1.75 |
| CI, L/min/m <sup>2</sup>                     | 2.50 ± 0.93 | 2.51 ± 0.96 |
| PVR, dyn·s·cm <sup>-5</sup>                  | 957 ± 493   | 802 ± 462   |
| PVRI, dyn·s·cm <sup>-5</sup> ·m <sup>2</sup> | 1760 ± 919  | 1505 ± 835  |

Data are presented as mean ± SD. BMI = body mass index; WHO FC = world health organization functional class; 6MWD = six-minute walk distance; GFR = glomerular filtration rate (Cochcroft); RAP = right atrial pressure; mPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; CO = cardiac output; CI = cardiac index; PVR = pulmonary vascular resistance; PVRI = pulmonary vascular resistance index; BSA = body surface area. \* NT-proBNP was measured in a subgroup of respectively n=72 and n=40.

**Table 2.** Baseline patient characteristics and Right Heart Catheterisation (RHC) and Cardiac Magnetic Resonance Imaging (CMR) measurements in male (n=26) and female (n=75) Pulmonary Arterial Hypertension

|                                   | Male n=26   | Female n=75 | p-value |
|-----------------------------------|-------------|-------------|---------|
| Age, years                        | 50 ± 19     | 47 ± 15     | 0.31    |
| Idiopathic, n (%)                 | 23 (88)     | 59 (79)     | 0.55    |
| Heritable, n (%)                  | 3 (12)      | 12 (16)     | 0.75    |
| Anorexigen, n (%)                 | 0 (0)       | 4 (5)       | 0.57    |
| BMI, kg/m <sup>2</sup>            | 27 ± 3      | 26 ± 6      | 0.34    |
| WHO FC, n (%)                     |             |             | 0.09    |
| Class I                           | 1 (4)       | 0 (0)       |         |
| Class II                          | 7 (27)      | 12 (16)     |         |
| Class III                         | 13 (50)     | 40 (53)     |         |
| Class IV                          | 5 (19)      | 23 (31)     |         |
| Comorbidities, n (%)              |             |             | 0.82    |
| 0                                 | 9 (35)      | 25 (33)     |         |
| 1                                 | 6 (23)      | 26 (35)     |         |
| 2                                 | 8 (31)      | 12 (16)     |         |
| ≥ 3                               | 3 (12)      | 12 (16)     |         |
| 6MWD, m                           | 388 ± 189   | 353 ± 150   | 0.40    |
| 6MWD, % predicted                 | 62 ± 27     | 61 ± 23     | 0.82    |
| Creatinine, mmol/L                | 110 ± 27    | 94 ± 17     | 0.001   |
| GFR, ml/min                       | 88 ± 31     | 75 ± 19     | 0.01    |
| NT-proBNP, ng/L*                  | 1414 ± 1668 | 1887 ± 1913 | 0.34    |
| RHC                               |             |             |         |
| RAP, mmHg                         | 10 ± 6      | 9 ± 5       | 0.11    |
| mPAP, mmHg                        | 53 ± 15     | 57 ± 13     | 0.29    |
| PCWP, mmHg                        | 8 ± 4       | 8 ± 5       | 0.65    |
| CO, L/min                         | 4.73 ± 1.63 | 4.55 ± 1.63 | 0.61    |
| PVR, dyn·s·cm <sup>-5</sup>       | 903 ± 545   | 963 ± 473   | 0.61    |
| Acute vasoreactivity <sup>#</sup> | 3/23 (13%)  | 7/66 (11%)  | 0.71    |
| CMR                               |             |             |         |
| RVEDV, ml                         | 177 ± 68    | 137 ± 41    | 0.001   |
| RVEDVI, ml/m <sup>2</sup>         | 89 ± 36     | 76 ± 21     | 0.03    |
| RVESV, ml                         | 124 ± 54    | 93 ± 35     | 0.001   |
| RVESVI, ml/m <sup>2</sup>         | 62 ± 28     | 52 ± 19     | 0.04    |
| RVEF, %                           | 31 ± 13     | 33 ± 11     | 0.44    |
| RVSV, ml                          | 53 ± 30     | 44 ± 19     | 0.38    |
| RVSVI, ml/m <sup>2</sup>          | 27 ± 17     | 25 ± 10     | 0.38    |
| RV mass, g                        | 104 ± 41    | 81 ± 28     | 0.009   |
| RV mass / RVEDV, g/ml             | 0.64 ± 0.31 | 0.62 ± 0.23 | 0.75    |

Data are presented as mean ± SD. BMI = body mass index; WHO FC = world health organization functional class; 6MWD = six-minute walk distance; GFR = glomerular filtration rate; RAP = right atrial pressure; mPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; CO = cardiac output; PVR = pulmonary vascular resistance; RVEDV = right ventricular end-diastolic volume; RVESV = right ventricular end-systolic volume; RVEF = right ventricular ejection fraction; RVSV = right ventricular stroke volume. CMR volumes are also provided indexed for body surface area. \* NT-proBNP was measured in a subgroup of n=20 males and n=52 females. <sup>#</sup> Acute vasoreactivity was measured in a subgroup of n=66 females and n=23 males. RV mass / RVEDV is a measure of relative RV wall thickness.



**Table 3.** Pulmonary arterial hypertension medical treatment regimens in respectively men and women.

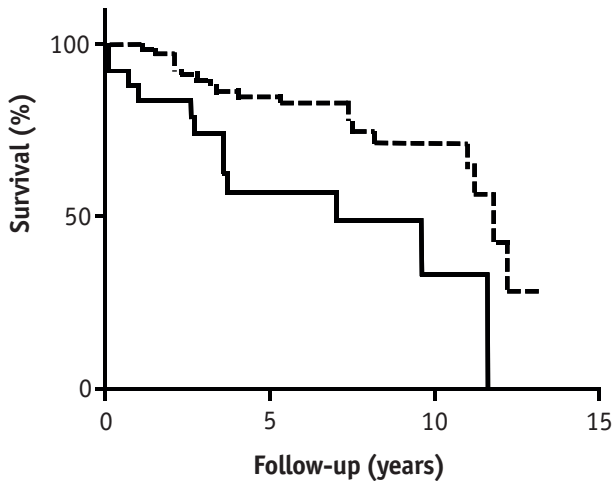
|  | Male (n=26) | Female (n=75) | p-value |
|--|-------------|---------------|---------|
| First-line therapy:                            |             |               |         |
| Prostacyclin                                   | 4 (15%)     | 23 (31%)      | 0.20    |
| ERA  | 13 (50%)    | 30 (40%)      | 0.74    |
| PDIE5  | 4 (15%)     | 9 (12%)       | 0.74    |
| ERA + PDIE5                                    | 2 (8%)      | 3 (4%)        | 0.60    |
| ERA + Prostacyclin                             | 0 (0%)      | 3 (4%)        | 0.57    |
| Ca <sup>2+</sup> blocker                       | 3 (12%)     | 7 (9%)        | 0.71    |
| Add-on therapy:                                |             |               |         |
| ERA + PDIE5 add-on                             | 4 (15%)     | 17 (23%)      | 0.58    |
| ERA + Prostacyclin add-on                      | 1 (4%)      | 1 (1%)        | 0.45    |
| Prostacyclin + PDIE5 add-on                    | 0 (0%)      | 2 (3%)        | 1.00    |
| PDIE5 + Prostacyclin add-on                    | 1 (4%)      | 0 (0%)        | 0.26    |
| Ca <sup>2+</sup> blocker + PDIE5 add-on        | 0 (0%)      | 1 (1%)        | 1.00    |
| Ca <sup>2+</sup> blocker + Prostacyclin add-on | 0 (0%)      | 2 (3%)        | 1.00    |
| Switch:  |             |               |         |
| From ERA to PDIE5                              | 1 (4%)      | 3 (4%)        | 1.00    |
| From Ca <sup>2+</sup> blocker to Prostacyclin  | 0 (0%)      | 2 (3%)        | 1.00    |

ERA = endothelin receptor antagonist. PDIE5 = phosphodiesterase type 5 inhibitor. Data are presented as number of patients n (% within sex).

### *Survival and secondary treatment outcomes*

In the 101 patients included median (IQR) follow-up time was 5.7 (2.5 to 8.1) years, and there were 26 deaths and 5 lung transplants. In males, cumulative transplant-free survival was 84% at 1 year and 57% at 5 years. In females, survival was 100% at 1 year and 85% at 5 years (logrank  $p=0.002$ ; HR 3.04; 95% CI 1.45-6.41; figure 1). The association between sex and survival after adjustment for confounders in multivariate analysis remained (HR 7.21; 95% CI 4.18-12.43;  $p<0.001$ ). The confounders retained in the final model were height, GFR and WHO functional class.

Male patients had higher NTproBNP, lower 6MWD and more severe functional class at follow-up in basic (table 4) and covariate-adjusted (table 5) models.



**Figure 1.** Transplant-free survival in male (solid line) and female (dashed line) patients with pulmonary arterial hypertension starting first-line pulmonary arterial hypertension specific therapies.

#### *RHC hemodynamics and CMR*

RHC showed no significant differences in treatment response associated with sex (table 4 and 5). Median PVR changes (IQR) were  $-78$  ( $-523$  to  $+10$ )  $\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$  in males and  $-165$  ( $-436$  to  $+92$ )  $\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$  in females.

Eighty patients had baseline and follow-up CMR performed. Reasons for not performing follow-up measurements in males were: patient deceased  $n=3$ , patient follow-up  $< 1$  year  $n=3$ , patient too disabled to undergo CMR  $n=3$ , unknown  $n=1$ . In females these were patient refusal  $n=4$ , patient follow-up  $< 1$  year  $n=3$ , patient too disabled  $n=2$ , psychiatric disorder  $n=1$  and technical CMR problem  $n=1$ . Corrections for missing follow-up measurements were made by multiple imputation.

After the baseline assessment, RVEF decreased in males (median, IQR)  $-1.0$  ( $-11.9$  to  $+6.9$ ) % and increased in females  $+3.6$  ( $-3.0$  to  $+13.0$ ) %. Table 4 and 5 depict results of univariate and multivariate analysis of sex difference in CMR changes. Calculated RVEF change corrected for confounders was  $-1.8 \pm 6.5$  % in males and  $+5.3 \pm 5.4$  % in females ( $p<0.001$ ).

#### *Mediator analysis*

Step 1 and step 2 of the mediator analysis were reported above. In step 1 sex was confirmed as an independent predictor of survival. In step 2 sex was confirmed as an independent predictor of RVEF change. Results of step 3 are reported in table 6, which shows the results of cox regression for transplant-free survival with sex

**Table 4.** Results of linear regression of sex differences in right heart catheterisation (RHC), MRI and other secondary treatment outcome parameters corrected for the baseline value.

| parameter                   | Difference for men vs. women<br>in follow-up measure after<br>adjustment for baseline | 95% CI         | p-value |
|-----------------------------|---|----------------|---------|
| NT-proBNP, ng/L             | +1385   | +482 to +2288  | <0.01   |
| 6MWD, m                     | -71   | -123 to -19    | <0.01   |
| Creatinine, mmol/L          | +17   | +6 to +29      | <0.01   |
| GFR, ml/min                 | -5  | -11 to +1      | 0.12    |
| WHO FC                      | +1.4  | +0.4 to +2.3   | <0.01   |
| Heart rate, beat/min        | +3  | -7 to +13      | 0.56    |
| RAP, mmHg                   | +2  | -1 to +6       | 0.17    |
| mean PAP, mmHg              | +1  | -7 to +9       | 0.81    |
| Cardiac output, L/min       | +0.2  | -1 to +1       | 0.78    |
| Stroke volume, ml           | -4  | -19 to +11     | 0.59    |
| PVR, dyn·s·cm <sup>-5</sup> | -60   | -301 to +182   | 0.63    |
| RVEF, %                     | -8.1  | -14 to -2      | <0.01   |
| RVEDV, ml                   | +11.9   | -5 to +29      | 0.18    |
| RVESV, ml                   | +13.8   | -2 to +30      | 0.09    |
| RVSV, ml                    | -5.5  | -14 to +3      | 0.19    |
| RV mass, g                  | +2.9  | -12 to +18     | 0.70    |
| RV mass / RVEDV, g/ml       | +0.04   | -0.09 to +0.16 | 0.57    |

b = coefficient for sex (male = 1; female = 0). 6MWD = 6-minute walk distance; GFR = glomerular filtration rate; RAP = right atrial pressure; PAP = pulmonary artery pressure; PVR = pulmonary vascular resistance; RVEF = right ventricular ejection fraction; RVEDV = right ventricular end-diastolic volume; RVESV = right ventricular end-systolic volume; RVSV = right ventricular stroke volume.

and the baseline value of the potential mediator. The B coefficient of sex changed substantially after RVEF follow-up measurements were added to the equation and significance of sex as predictor of transplant-free survival was lost, thus showing evidence that the impact of sex on survival was mediated through RVEF at follow-up. There is no evidence for mediation through PVR changes as the B coefficient for sex remains similar in the cox regression formula with sex and baseline PVR compared to the formula with sex, baseline PVR and follow-up PVR. The amount of change in B for sex after adding follow-up values of RVEF or PVR to the Cox regression equation gives a sense of how much of the variance in outcome associated with sex is explained by changes of each hemodynamic parameter. In the basic model, 42.8 % of the effect of sex on survival was mediated through RVEF. After adjustment for confounders this was 39.0 %.

**Table 5.** Results of multivariate analysis\* of sex specific right heart catheterisation (RHC), MRI and other treatment outcome parameter changes compared to baseline.

| parameter                   | Difference for men vs. women<br>in follow-up measure after<br>adjustment for baseline and<br>confounders | 95% CI         | p-value |
|-----------------------------|--|----------------|---------|
| NT-proBNP, ng/L             | +1385  | +482 to +2288  | <0.01   |
| 6MWD, m                     | -70  | -127 to -12    | 0.02    |
| Creatinin, mmol/L           | +14  | +3 to +25      | 0.01    |
| GFR, ml/min                 | -6   | -13 to 0       | 0.05    |
| WHO FC                      | +1.9   | +0.9 to +3.0   | <0.001  |
| Heart rate, beat/min        | +5   | -7 to +17      | 0.42    |
| RAP, mmHg                   | +2   | -1 to +6       | 0.25    |
| mean PAP, mmHg              | +2   | -8 to +11      | 0.73    |
| Cardiac output, L/min       | +0.0   | -1 to +1       | 0.99    |
| Stroke volume, ml           | -7   | -24 to +11     | 0.45    |
| PVR, dyn·s·cm <sup>-5</sup> | -35  | -337 to +267   | 0.82    |
| RVEF, %                     | -7.2   | -13 to -1      | 0.02    |
| RVEDV, ml                   | -0.4   | -19 to +18     | 0.97    |
| RVESV, ml                   | +5.2   | -13 to +23     | 0.58    |
| RVSV, ml                    | -9.5   | -19 to 0       | 0.04    |
| RV mass, g                  | +3.8   | -13 to +21     | 0.67    |
| RV mass / RVEDV, g/ml       | +0.09  | -0.05 to +0.24 | 0.22    |

\* Multivariate analysis results showing the coefficient b for sex (male = 1; female = 0) corrected for potential confounding by age, weight, height, number of comorbidities, baseline RVEF, GFR, PVR, WHO functional class and type of PAH specific medical therapy initiated.

6MWD = 6-minute walk distance; GFR = glomerular filtration rate; RAP = right atrial pressure; PAP = pulmonary artery pressure; PVR = pulmonary vascular resistance; RVEF = right ventricular ejection fraction; RVEDV = right ventricular end-diastolic volume; RVESV = right ventricular end-systolic volume; RVSV = right ventricular stroke volume.

**Table 6.** Results from cox-regression for transplant-free survival with respectively sex and the baseline measurement and subsequently sex, the baseline measurement and the follow-up measurement for respectively RVEF and PVR. Crude analysis (A) and analysis including corrections for confounders (B) is reported.\*

|                         | <b>B</b>     | <b>Exp (B)</b> | <b>95% CI of Exp (B)</b> | <b>p-value</b> |
|-------------------------|--------------|----------------|--------------------------|----------------|
| <b>A</b>                |              |                |                          |                |
| Gender (male vs female) | <b>1.029</b> | 2.80           | 1.33 - 5.91              | <b>0.007</b>   |
| Baseline RVEF           | -0.05        | 0.95           | 0.92 - 0.99              | 0.007          |
| Gender                  | <b>0.589</b> | 1.80           | 0.81 - 4.01              | <b>0.15</b>    |
| Baseline RVEF           | -0.01        | 0.99           | 0.95 - 1.04              | 0.81           |
| Follow-up RVEF          | -0.07        | 0.94           | 0.89 - 0.98              | <b>0.006</b>   |
| <b>B</b>                |              |                |                          |                |
| Gender                  | <b>1.397</b> | 4.04           | 2.50 - 6.54              | <b>0.004</b>   |
| Baseline RVEF           | -0.05        | 0.95           | 0.93 - 0.97              | 0.009          |
| Gender                  | <b>0.852</b> | 2.34           | 0.93 - 5.92              | <b>0.07</b>    |
| Baseline RVEF           | -0.01        | 0.99           | 0.95 - 1.04              | 0.76           |
| Follow-up RVEF          | -0.07        | 0.94           | 0.89 - 0.98              | <b>0.006</b>   |
| <b>A</b>                |              |                |                          |                |
| Gender                  | <b>1.11</b>  | 3.04           | 2.08 - 4.45              | <b>0.003</b>   |
| Baseline PVR            | 0.00         | 1.00           | 1.00 - 1.00              | 0.95           |
| Gender                  | <b>1.20</b>  | 3.31           | 1.53 - 7.16              | <b>0.002</b>   |
| Baseline PVR            | 0.00         | 1.00           | 1.00 - 1.00              | 0.53           |
| Follow-up PVR           | 0.00         | 1.00           | 1.00 - 1.00              | 0.12           |
| <b>B</b>                |              |                |                          |                |
| Gender                  | <b>1.51</b>  | 4.52           | 1.83 - 11.18             | <b>0.001</b>   |
| Baseline PVR            | 0.00         | 1.00           | 1.00 - 1.00              | 0.84           |
| Gender                  | <b>1.476</b> | 4.38           | 1.77 - 10.84             | <b>0.001</b>   |
| Baseline PVR            | 0.00         | 1.00           | 1.00 - 1.00              | 0.48           |
| Follow-up PVR           | 0.00         | 1.00           | 1.00 - 1.00              | 0.19           |

\* RVEF = right ventricular ejection fraction; PVR = pulmonary vascular resistance.

## Discussion

Our data confirmed previous findings of worse outcome in males.<sup>6</sup> This survival difference was not associated with either baseline characteristics or differences in responsiveness of the pulmonary vascular bed to therapy, but rather differences in RVEF after starting medical therapies. BNP changes are correlated with RV strain and RVEF measured by CMR and the BNP differences found in our study further support our CMR findings.<sup>17-19</sup> In an earlier study RVEF change difference between survivors and non-survivors in PAH was 8%, further illustrating that the difference found in our study is clinically meaningful.<sup>20</sup>

Sex differences have been well documented in diseases of the left ventricle. In the Framingham study, worse survival was observed in male heart failure patients.<sup>21</sup> Systolic heart failure is predominantly found in men whereas women rather present with heart failure with preserved ejection fraction.<sup>22</sup> In analogy female pressure loaded hearts showed more preserved ejection fractions in aortic stenosis.<sup>23</sup> In a recent study of hypertensive patients left ventricular mass variance explained by arterial blood pressure was much higher in females. This could be interpreted as further evidence of better cardiac adaptation in females.<sup>24</sup>

Little is known about sex differences in disease of the right ventricle. Healthy women have lower RV mass, smaller RV volumes, and higher RVEF than men.<sup>25</sup> Ventetuolo et al. showed an association between higher estradiol levels and improved RVEF in women and an association between increased androgen levels and increased right ventricular mass and right ventricular volumes.<sup>26</sup> In a rodent model testosterone and oestradiol both caused pulmonary vasodilation.<sup>27</sup> In male mice testosterone affected RV hypertrophic stress response after pulmonary artery banding through increased myocyte size and increased fibrosis. Testosterone deprivation through castration improved survival in these mice.<sup>28</sup> In addition estrogen and estrogen receptor agonist therapy restored RV structure and function in a rodent model of monocrotaline induced PH.<sup>29</sup>

Our study found no differences in pulmonary vascular responses to PAH specific medications. Hitherto no other studies in humans reported on sex differences in pulmonary vascular response. We found no sex differences in cardiac output, and this further points out the problems with only looking at resting cardiac output, rather than at RV structure and RV systolic function (RVEF). During disease progression resting cardiac output can be maintained through an increased heart rate. In addition stroke volume can be relatively preserved through the Starling mechanism. However, in progressive RV dilation RVEF will decrease and RVEF may be a more sensitive parameter for disease progression.<sup>2</sup> It can not be ruled out that CO differences do occur upon exercise.

Our study has some limitations. Not all patients evaluated at our center were included. While those included appeared similar to those excluded, selection bias could still be possible. We attempted to account for a variety of confounders, however we cannot exclude residual or unmeasured confounding. There were some missing data; we used multiple imputation to allow inclusion of all subjects in the study sample in all

analyses. Finally, this is an observational study, preventing us from confirming causality, however the use of sex as our exposure and prospective reassessments of RV function support causal inferences. We only studied the idiopathic, heritable and anorexigen associated form of PAH, so these findings may not be generalizable to other forms of PAH. However sex differences in survival are also reported in connective tissue disease associated PAH<sup>30</sup>, although in associated PAH the survival difference was limited to elderly patients.<sup>9</sup> Since RVEF could explain 40 % of the observed survival difference, other factors must contribute. However, these factors cannot be identified through our study, as the small patient number prohibits further exploratory analysis.

In conclusion our study suggests a sex difference in cardiac adaptation to treatment with long-term improvements in RVEF in women, but not in men. Mediator analysis suggests this different cardiac adaptation may cause decreased survival in males. To further improve treatments the pathophysiology of sex differences in cardiac response to medical therapies should further be elucidated. Evidence for differences in cardiac responses in associated forms of PAH should be studied. Furthermore, the role of sex hormones, and the potential of substances targeting sex-specific pathways, such as estrogen receptor agonists should be further evaluated.<sup>29</sup>

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